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Title ENDOCRINOLOGY OF SEX-LINKED-RECESSIVE DWARF
WHITE LEGHORN CHICKENS, GALLUS DOMESTICUS.

WITH SPECIAL REFERENCE TO THE THYROID

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A comparison of the various endocrine glands, in addition to other anatomical measurements, was made on 19 normal and 21 dwarf 2-3-month-old females and 20 normal and 20 dwarf 7-13-month-old females of the White Leghorn breed. At both ages, dwarfs weighed significantly less and had significantly shorter shanks than normals. Comb weight and comb area (L X H) were significantly larger in normals than in dwarfs 7-13 months of age, but no significant difference was found in the 2-3 month age group. Ovaries in the 2-3 month age group were heavier in normals than in dwarfs, due to greater gonadotropin secretion in relation to body size. The divergent results in weight of the hypophyses on an absolute and a relative-to-body-weight basis may be attributed to the algebraic sum of
synthesis storage and liberation of hormones by this gland. Also, absolute and relative weights of the adrenals did not always coincide in both age groups, which may be attributed to environmental factors influencing the release of ACTH from the hypophysis. The normals had significantly heavier thyroids than dwarfs, on an absolute and on a relative weight basis at both ages, which does not necessarily indicate a hyper- or hypothyroid condition in the latter group of birds. Normals secreted significantly more thyroxine than dwarfs at 7-13 months of age; whereas, no significant difference was found between these two types of chickens at 2-3 months of age, as determined by epithelial cell height. The significantly smaller colloid and follicle diameter found in thyroids of dwarfs than of normals may be attributed to the lower gland weights in the former group.

Bioassay of the hypophyses from these chickens was conducted in immature, hypophysectomized, female rats. Dwarfs did not differ significantly from normals in STH activity, according to the tibia test, body weight gain and tail length. Greater epiphyseal plate responses were obtained from hypophyses of growing chickens. Thymus weight varied with dosage, which is attributed to several hormones acting on this gland. The normals had a significantly higher hypophyseal ACTH content than dwarfs 2-3 months of age, which is believed to be due to the hormone content of the hypophyses at the time of removal, rather than to dwarfism. Also, the younger
birds were observed to have a higher ACTH content than older birds, which may be due to storage of this hormone in the hypophysis during the growing period. Dwarfs did not differ significantly from normals in gonadotropic activity at both ages, as evaluated by ovary, oviduct and uterus weight response in the assay rats. However, the older birds were observed to have higher gonadotropic activity than younger birds, as determined by ovary, oviduct and uterine weight and vaginal openings in the rats. No significant difference in FSH content of the hypophyses of dwarfs and normals of both ages was found, using ovarian follicle diameter. Observation of the interstitial cells in the rat ovaries revealed no difference between dwarfs and normals at both age classifications in ICSH content. No significant difference in TSH activity between dwarfs and normals within each age group was found, based on thyroid weight in the rats. Also, dwarfs did not appear to differ from normals of both ages in measurements on epithelial cell height and colloid and follicle diameter. Lack of appreciable differences between the treated rats and the controls in these measurements on the thyroids would indicate that rats did not respond to avian TSH.
ENDOCRINOLOGY OF SEX-LINKED-RECESSIVE DWARF WHITE LEGHORN CHICKENS, GALLUS DOMESTICUS, WITH SPECIAL REFERENCE TO THE THYROID

by

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ENDOCRINOLOGY OF SEX-LINKED-RECESSIVE DWARF WHITE LEGHORN CHICKENS, *GALLUS DOMESTICUS*, WITH SPECIAL REFERENCE TO THE THYROID

INTRODUCTION

Poultrymen are constantly striving to reduce the cost of egg production. In past years, costs of production have been decreased by increased labor efficiency and increased egg production. Automatic feeding and egg gathering equipment in addition to the use of cages have decreased the amount of labor required in commercial poultry operations. Other possibilities of reducing costs are under investigation. It would seem that a reduction in body size of the layer would be economically desirable and biologically possible.

Historically, poultry breeders have selected for heavier birds because this was thought to mean greater stamina and increased egg size. There was no conclusive evidence for the relationship between body size and economic performance until the investigation of Nordskog (1962), who found that selection for large egg size had little influence on body weight; whereas, selection for small egg size caused a large decrease in body weight.

Since the beginning of the broiler industry, the value of the spent layer has decreased; therefore, attention has been given to the possibility of reducing the depreciation of the laying flock through
small body size. Production costs could be reduced by lowering feed costs for maintenance and growth of layers and also by reducing floor space requirements. Results from random sample laying tests have shown that smaller strains of chickens require less feed to produce a dozen eggs because of lower feed requirements for body maintenance.

The factor of major concern is the biological relationship between body size and egg production and egg characteristics.

At the present time, breeding organizations are selecting layers for small body size. One possibility of making faster progress is by taking advantage of a major reduction in body size such as is available in the sex-linked-recessive White Leghorn dwarf that has been under investigation since 1958 at the Oregon Agricultural Experiment Station.

To allow for a better utilization of the mutation, it appeared essential to obtain a better understanding of its physiology, particularly its endocrinology; hence the following investigations on the endocrinology of the dwarf chicken.
LITERATURE REVIEW

In this review of literature, attention has been given to different hereditary types of dwarfism, not only in Aves but also in Mammalia, in hopes of better understanding sex-linked-recessive dwarfism in chickens.

Dwarfism in Avian and Mammalian Species

Autosomal Recessive Dwarfism in Chickens

Landauer (1929) is apparently the first to describe a Rhode Island Red dwarf homozygous for an autosomal recessive gene subsequently symbolized by Hutt (1949) as \( \text{td} \) (Thyrogenous dwarfism). Landauer found this dwarf exhibited very little temperament. The plumage appeared ragged due to dryness of the feathers and skin. The tongue was malformed and the eyes were large and protruding. The comb, wattles, ovary and oviduct were underdeveloped. The tarso-metatarsus (shank) was reduced in length. The thyroids were twice the normal size and consisted mostly of aplastic tissue with very small follicles lacking in colloid. Landauer attributed this type of dwarfism to a hypothyroid condition similar to Myxoedema infantilis in man.

Mayhew and Upp (1932) reported on the same type of dwarf and observed in a few cases that the outer toe was curled backwards,
which may be evident at hatching time. These investigators observed swollen tissues around the eyes giving these the appearance of protruding. The longest a dwarf of this genetic make-up has been known to live is 83 weeks.

Upp (1932) implanted one hypophysis from White Leghorn cockerels subcutaneously for 15 days into one Rhode Island Red dwarf 161 days of age. This treatment was followed by implanting one hypophysis daily for 15 days from adult Rhode Island Red males. A second dwarf, 161 days of age, received thyroid tissue implants instead of hypophyses for the same period of time. No response was obtained in either case.

**Incomplete Recessive Dwarfism in Chickens**

Van Tienhoven and Cole (1962) and Cole (1966) have investigated the endocrine system of polygenic dwarfs. Because of the unusually large amounts of abdominal and subcutaneous fat present, these dwarfs were referred to as obese and could be recognized at 6-8 weeks of age.

Van Tienhoven and Cole (1962) found that 25-28-week-old pullets had significantly heavier hypophyses and smaller combs and
thyroids than the normals\(^1\). The thyroids were histologically abnormal, making their weight an approximate measure. The ovarian follicles were less than 5 mm in diameter and the oviduct lacked development. Bioassay of adenohypophyseal (anterior pituitary) homogenate revealed no significant difference in gonadotropic potency between obese and normals, based on testicular response of cockerels. The evaluation of thyroid stimulating hormone (TSH) activity determined by epithelial cell height was significantly lower in normals than in obese pullets.

Graded levels of TSH did not produce a response in comb size, ovary weight, oviduct weight and feather regeneration in 7-8-month-old obese hens. A level of \(1.6 \mu g\) of dl-thyroxine per kilogram of body weight produced a significant increase in comb size, ovary and oviduct weight. Dl-thyroxine also stimulated the growth of new feathers in obese hens. Another part of these investigations involved the use of pullet chicks that received 10 gm of thyroprotein (iodinated casein) per 100 lbs of chick starter ration. This treatment did not significantly increase body and ovarian weight in these obese chicks. This type of dwarfism is attributed to a primary hypothyroid condition which is in accord with the observations made earlier by Payne

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\(^1\) The word normal is used reluctantly because of the difficulty in defining it. Since there seems to be no suitable alternative, normal will refer to a chicken with characteristics of the commercial laying strains.
(1944). He found the presence of thyroidectomy cells (T-cells) in 6-month-old dwarfs; however, the genetic make-up of these birds was not reported.

According to Cole (1966), the thyroids of this dwarf obese chicken are flat, elongated and spindle shaped. In some cases, one or both glands are absent. The chick feathers are normal but after the juvenile molt at about 8 weeks, the plumage becomes silky. Frequently, as the dwarf reaches sexual maturity, long contour downy feathers are observed. The mature female will begin to lay before the comb has attained maximum growth and color. The facial region is pale. In the male, comb growth and coloration are about normal. The injection of 0.4 IU of TSH per kilogram of body weight stimulated the thyroids in some of the obese chickens. However, the exogenous source of TSH will not maintain the integrity of the thyroid. Egg production and egg size were improved by feeding protamone (iodinated casein) as were fertility and hatchability. These findings provide further evidence for a hypothyroid condition in obese dwarfs.

Sex-Linked-Recessive Dwarfism in Chickens

Hutt (1959) was the first investigator to report on sex-linked-recessive dwarfism in a New Hampshire. The gene (dw) appears to be completely recessive in the heterozygous male. These dwarfs
are proportional in body characteristics. The \textit{dw} gene reduces the body weight of homozygous males about 43 percent below normal and that of hemizygous females about 30 percent.

According to Hutt (1959), there was no significant difference in hatching weight between dwarfs and normals. The normal females were significantly heavier than dwarfs at two weeks of age. Also, the normals were significantly heavier than dwarfs at six weeks of age, and the latter continue to grow at a slower rate thereafter. Dwarfism significantly delayed the onset of egg production. During the three months of highest egg production, the dwarfs laid 12.7 to 18.6 percent fewer eggs than their normal sisters. Fertility, hatchability and viability were found to be the same in dwarfs as in normals. In this investigation, Hutt found a reduction in the three leg bones, especially the tarso-metatarsus.

Bernier and Arscott (1960) found that dwarf White Leghorn hens weighed 63 percent as much and consumed 84 percent as much feed per dozen of 24 oz eggs as their normal-size sisters. The dwarfs laid 18 percent fewer eggs that weighed 10 percent less per dozen.

The dwarfs produce eggs with thinner egg shells than their normal-size sisters, according to Bernier and Arscott (1960) and Arscott, Rachapaetayakom and Bernier (1961, 1962). These investigators have found that increased calcium in the ration improved
shell thickness and egg production in the dwarfs. Arscott and Bernier (1966) have observed the calcium requirement to be 2.75 percent of the total ration for normals and 3.80 percent for dwarfs.

Bernier and Arscott (1966) compared the growth and feed requirements of dwarf pullets to those of their normal-size sisters from day-old to 23 weeks of age. The results from 700 birds of each type are presented in Appendix Table I. Mortality to 17 weeks of age was not affected by dwarfism. The dwarfs reached 25 percent production two weeks later than the normals. Shank length of normals and dwarfs at 8 and 17 weeks of age was 8.3 and 10.3 cm, respectively, for the former and 7.1 and 8.2 cm, respectively, for the latter.

Investigation of the thyroid and hypophysis of sex-linked dwarfs has been conducted by van Tienhoven et al (1966). These workers added 0.04 percent protamone to the diet of dwarf and normal (K-line) chicks from day-old to 20 weeks of age. Protamone did not correct the dwarf condition. The adenohypophysis was smaller in dwarfs than in normals. The absolute thyroid weight and percent colloid were lower in dwarfs than in normals. Body weight and shank length were in accord with those reported by Hutt (1949), 1953, 1959) and Bernier and Arscott (1966). A bone-ash determination of the tibia-tarsus revealed no significant difference between the dwarf and the normal birds at 14 to 16 weeks of age.
Autosomal Recessive Dwarfism in Mice

Research with dwarf mice has been centered mainly around two strains, according to Bartke (1964). The Snell strain (dw) and the Ames strain (df) are indistinguishable as to postnatal retardation of growth, final body size, and body proportions, but some of the former strain become obese and exhibit myxoedematous changes of the skin at six months of age. The thyroids in both of these dwarfs are reduced in size and these animals exhibit sluggishness and a low basal metabolic rate (BMR). Both strains are sterile. Cytological studies reveal the lack of acidophils and a reduction in the number of thyrotrophs in the adenohypophysis. These two types of dwarfs have similar hormone deficiencies but are physiologically different in response to somatotropic hormone (STH) and TSH. Greater responses to STH and TSH were exhibited by the df dwarfs than by the dw dwarfs. Somatotropic hormone and TSH together produce a greater response than STH alone. There is only a slight response in the testes of these dwarfs but nevertheless most of the df males and some of the dw males become fertile. The ovaries are increased in size and become functional after treatment with STH and TSH. The thyroids are not stimulated by thyroxine administration.

A recent investigation by Viola-Magni (1965) has shown that
in the Snell dwarf each motor neuron innervates more muscle fibers than in the normal mouse resulting in reduced activity with fewer quick coordinated movements.

**Sex-Linked Dwarfism in the Rat**

Lambert and Sciuchetti (1935) have reported on dwarfism in the rat resulting from a disturbance in one of the endocrine glands. These dwarfs lack libido and all have proven entirely sterile. One of the most interesting features of the syndrome is that males and females do not become differentiated in size.

**Incomplete Recessive Dwarfism in Rabbits**

Green, Hu and Brown (1934) have reported on an incomplete recessive dwarfism in rabbits, probably due to a hypophysis hypo-function in homozygotes and heterozygotes.

**Autosomal Recessive Dwarfism in Guinea-Pigs**

Sollas (1909) worked with a strain of simple recessive dwarf guinea-pigs. The dwarfs which did not die at an early age were sterile and half normal size. There were also alterations in the skeleton of these dwarfs.
Autosomal Recessive Dwarfism in Beef Cattle

Wriedt (1930), Ensminger (1955), Bogart et al (1962) and Clark et al (1963) have discussed many different types of dwarfism in beef cattle, apparently caused by a simple autosomal recessive gene. One of the most prominent types is a short-headed dwarf resulting from a hypofunction of the thyroid.

Bioassay Studies

Bird Hypophyses in Intact and Hypophysectomized, Immature Rats

Schlumberger and Rudolph (1959) found that transplantation of parakeet Melopsittacus undulatus hypophyseal tumors in hypophysectomized rats increased the width of the epiphyseal plate in the tibia test for STH. Dr. C. H. Li, in a personal communication with Schlumberger and Rudolph, stated that 0.5 mg of chicken hypophyses did not increase the width of the epiphyseal plate in hypophysectomized female rats. Solomon and Greep (1959) conducted a comparative study using the tibia test. Two-thousand micrograms of acetone-dried hypophyses from the frog, rabbit, cat and horse and adeno-hypophyses from the whale produced a significant growth response. At the same dosage, turtle and chicken hypophyseal extracts induced a measurable but non-significant growth response; whereas, shad hypophyses failed to produce epiphyseal response. According to
Hazelwood and Hazelwood (1961), adenohypophyseal homogenate from 9-12-week-old White Rocks contained one-eighth of the growth-stimulating principles found in an equivalent amount of rat hypophyseal extract. Moudgal and Li (1961) reported that 0.5 mg of lyophilized chicken hypophyses induced growth response according to the tibia test.

One of the earliest experiments for evaluating sex hormone potency of chicken endocrine glands with the rat as the bioassay animal was conducted by Allen et al (1924). Ovaries were removed and eggs were collected from the oviducts of hens. Extracts of small and medium sized ovarian follicles produced cornification of the vaginal epithelium in spayed rats. Extracts of full size follicles and extracts of albumen and yolks from eggs taken from the oviduct and from freshly laid eggs as well as 5, 10, and 14-day-old embryos plus membranes did not produce vaginal cornification in the rats. Leonard (1938) discovered that graded levels of acetone-dried chicken hypophyses from different age groups not segregated according to sex increased ovarian and uterine weights of immature hypophysectomized female rats. In this same investigation, adrenal weight was increased and histological studies revealed cortex enlargement. The thyroids were not stimulated. Acetone-dried hypophyses from seven different age groups of chickens were assayed for gonadotropic potency by Phillips (1942). Gonadotropic activity was evaluated by
changes in the vaginal epithelium. The chickens were ranked in descending order according to gonadotropic potency as follows:

(1) capons; (2) springs, 2.5-3.5 lbs; (3) roasters, 3.5-6.0 lbs; (4) turkeys; (5) pullets approaching sexual maturity; (6) fowl, medium to poor fecundity; and (7) fowl, good production. Rat ovarian weight was found to be inaccurate for determining gonadotropic potency.

Myer, Mellish and Kupperman (1939) prepared hypophyseal homogenate from male and female chickens. Hypophysectomized and intact female rats were injected with 50, 100, 200, 300, 500 and 700 mg of hypophyses from both sexes. An additional 900 mg was administered from female chickens. Male hypophyses produced a greater ovarian weight response than those from females. However, at the higher levels, it appeared that the gonadotropic effect was greater in the intact animals, but at the lower levels response was approximately the same. It is probable that the ovaries have to develop to a certain point before the hormones from the hypophysis enter into the reaction. Also, since a discrepancy in ovarian weight between the two types of rats appeared at the point where lack of tolerance to the material was observed, the large amount of hypophysis, in addition to the disturbed metabolism of the operated rats, may have prevented an increase in ovarian weight. The adrenocorticotropic hormone (ACTH) content of 500 mg of homogenate completely replaced adrenal weight in hypophysectomized rats. There were no
sex differences. Riley and Fraps (1942a) assayed 10 mg of acetone-dried adenohypophyses from Rhode Island Red males 7-8 months of age in immature intact rats. Complete vaginal cornification and extensive ovarian follicle response were obtained at this dosage. Herrick, McGibbon and McShan (1962) compared gonadotropic potency by assaying 10 mg of adenohypophyseal homogenate from chickens, turkeys and rats in immature female rats, 3-day-old turkey poults and 3-day-old chicks. An increase in testicular size in chicks was obtained from the hypophyses of each species, but the greatest increase was from chicken hypophyses. Only gonadotropins from rats stimulated the ovaries of the immature rats. According to Nelson, Norton and Nalbandov (1965), the ovarian ascorbic acid depletion (OAAD) method can be used satisfactorily for evaluation of interstitial-cell-stimulating hormone (ICSH) levels in plasma and in adenohypophyses of chickens. No signs of toxicity to plasma based on general behavior of the rats were observed.

**Turkey Hypophyses in Immature Intact Rats**

Witschi, Stanley and Riley (1937) evaluated the gonadotropic activity of 10, 25, 50 and 100 mg of acetone-dried hypophyses from 6-month-old male and female turkeys in immature, intact female rats. All levels except the 10 mg level increased ovarian weight. All levels increased uterine weight but had no influence in time of
vaginal opening. No difference in response could be attributed to the sex of the turkey.

**Chicken Hypophyses in the Salamander**

According to Stein (1934), the hypophysis or adenohypophysis of various age groups of chickens implanted or injected into the salamander will cause ovulation out of season in some of these amphibians. The thyroids of the salamander were stimulated by adult and chick hypophyseal administration.

**Chicken Hypophyses in Immature Intact Mice**

Zavodovsky (1929) found that implantation of a single chicken hypophysis under the skin of mice produced greater response in their sex glands than the dog hypophysis. Riley and Fraps (1942a) concluded that 21-day-old mouse uterine weight response was a practical and efficient index of the gonadotropic potency of acetone-dried adenohypophyses from male chickens. Further investigations by Riley and Fraps (1942b) revealed that 21-day-old mouse ovarian weight response can be used to determine the gonadotropic potency of acetone-dried adenohypophyses from hens with ovarian follicles in different stages of development. Breneman (1945) discovered that acetone-dried and fresh adenohypophyses from cockerels produced uterine weight and vaginal epithelium response in 23-day-old
female mice. However, the quantitative bioassay was unsatisfactory because in several instances there was little difference between cockerels, capons and pullets receiving 1, 2 and 3 hypophyses.

**Pigeon Hypophyses in the Immature Mouse**

Smith and Engle (1927) were unsuccessful in attempts to induce precocious sexual maturity in 17-day-old mice by transplantation of the adenohypophysis from 100-day-old pigeons.

**Hypophyses from Different Species in Birds**

Riddle and Schooley (1935) discovered that the testes of immature ring doves were stimulated by implanting hypophyses from 29-44-day-old rats. The testes of 5-7-day-old chicks were moderately stimulated by implanting hypophyses from castrated rats and the female hypophyses produced the greatest response. According to Herrick, McGibbon and McShan (1962), 10 mg of adenohypophysial homogenate from adult male rats stimulated the testes of chicks in the determination of gonadotropic activity. Riddle and Flemion (1928) found that a commercial glycerine extract of fresh bovine adenohypophyses increased testis size of 11 immature ring doves by fifty to several-hundred percent. Somatotropic hormone of mammalian origin had little effect on growth in young chickens according to Libby, Meites and Schaible (1955); Carter, Risner
and Yacowitz (1955); and Glick (1960). Blumenthal, Hsieh and Wang (1954) found that STH increased the length of the long bones in chick embryos. Fractionated sheep hypophyses caused ovulation of the first follicle in a clutch 6-8 hours prematurely in 10 of 20 treated layers, according to Fraps, Fevold and Neher (1947). Byerly and Burrows (1938) discovered that testes of day-old cockerels respond quickly to pregnant mare serum gonadotropin (PMSG). Follicular fluid from sows did not hasten sexual maturity in immature ring doves (Riddle and Tange, 1928). However, the oviducts of a few treated birds were increased in size. According to Foglia (1941), the adenohypophysis of the toad has higher thyrotropic than adrenotropic action in the chicken.
EXPERIMENTAL MATERIALS AND METHODS

A number of anatomical measurements were recorded on 19 normal and 21 dwarf females 2-3 months of age and 20 normal and 20 dwarf females 7-13 months of age. Birds were killed by decapitation after weighing. The comb was removed with scissors and weighed on a triple-beam balance. Measurements of height and length were noted and comb area determined by multiplying length times height \((L \times H)\) according to the technique used on live fowl by Jones and Lamoreux (1943). Shank length was determined by measuring the distance from the hock joint to the foot pad (Lerner, 1937).

The thyroids, adrenals, ovaries and hypophyses were removed under a dissecting microscope, trimmed free of adhering tissues and weighed on a Roller-Smith torsion balance. The thyroids were fixed in Bouin's solution for histological studies. The hypophyses were immediately frozen on dry ice, placed in vials and stored in a freezer for later bioassay.

Preparation of Chicken Hypophyseal Homogenate

The hypophyses from dwarf and normal chickens for each age were thawed, pooled and suspended in a volume of 0.9 percent physiological saline solution that would make the highest hypophyseal dosage. The glands were homogenized with a motor-driven
homogenizer. Aliquots of the homogenate were diluted with 0.9 percent saline solution for preparing dosage levels equivalent to 4, 2, 1 and 1/4 hypophyses.

**Assay Animals**

The biological assay of the chicken hypophyses was conducted with immature female rats of the Long-Evans strain, hypophysectomized at 26-28 days of age. These rats were maintained in a heated small-animal laboratory. Purina Rat Chow and drinking water were supplied ad libitum. There was a 14-day post operative period before treatments were started. The assay animals were injected intraperitoneally with 1.0 cc of homogenate once daily for four days. The control rats received 1.0 cc of saline solution. Twenty-four hours after the last injection, the rats were asphyxiated with chloroform.

**Evaluation of Hormone Potency**

Initial weights were determined prior to treatment and final weights were recorded at autopsy on the assay animals. Tail length was determined by measuring the distance from the anus to the tip of the tail. The adrenals, thyroids, ovaries, oviducts and uteri and thymus were removed under a dissecting microscope, trimmed free of adhering tissues and weighed on a Roller-Smith torsion balance. The sella turcica was examined for hypophyseal
fragments and if such fragments were present the rat was not used in the experiment. The thyroids and ovaries were fixed in Bouin's solution for histological studies.

The tibia test for STH involved the measurement of the width of the epiphyseal plate as proposed by Evans et al. (1943). At autopsy, the right tibia was removed and dissected free of adhering tissues. The proximal end of the tibia was split with a razor blade in a sagittal plane and fixed in 10 percent formalin. Prior to measurement, the bone halves were treated in the following order:

1. Running tap water for 1/2 hour
2. Acetone for 1 hour
3. Distilled water for 1/2 hour
4. Two percent silver nitrate \((\text{AgNO}_3)\) for 1-3 minutes
5. Distilled water with cut surface up under a continuous source of light for approximately 1 minute until calcified portion of the bone is sufficiently brown to clearly identify the epiphyseal plate.
6. Ten percent sodium thiosulfate \((\text{Na}_2\text{S}_2\text{O}_3)\) for 70 seconds
7. Distilled water after staining
8. Running tap water for 1/2 hour
9. Eighty percent ethanol and place in the dark.

With the use of a calibrated ocular micrometer, ten individual readings were taken across the epiphyseal plate of each tibia, then
averaged and converted to microns.

Evaluation of total gonadotropic activity is represented quantitatively by ovarian and oviduct and uterine wet weight responses, and qualitatively by vaginal opening. The diameter of non-atretic follicles was used to determine FSH activity (Evans et al., 1939). The ovaries were embedded in paraffin, sectioned with a microtome at 8 micra and stained with hematoxylin and eosin. Seven sections from one or both ovaries of each rat were mounted on triplicate slides. With the use of a calibrated ocular micrometer, the largest ovarian follicle for each rat was measured and the average also was calculated for each dosage. Interstitial cell repair as described by Simpson, Li and Evans (1942) was used to evaluate ICSH activity. The same histological preparations as described for FSH were used for this determination. The degree of interstitial cell repair is defined thus:

1. D (deficient)
   a. Nuclei small with chromatin in clumps
   b. Nuclei close together
   c. Cytoplasm not very evident

2. PR (partially repaired)
   a. Nuclei increased in size and slightly more rounded with the chromatin more evenly scattered
   b. Nuclei further apart and stained lighter with
hematoxylin
c. Cytoplasmic body increased in size and more
eosinophilic

3. R (repaired)
   a. Interstitial cells PR with increased response.

4. H (hypertrophy)
   a. Nuclei large with even distribution of chromatin
   b. Nuclei far apart and lightly stained
   c. Cytoplasmic body increased in size and highly
eosinophilic.

The method for histological evaluation of the secretory
activity of the thyroids from the chickens and assay animals was
developed in this investigation. The thyroids were embedded in
paraffin or paraplast, sectioned with a microtome at 8 micra and
stained with hematoxylin and eosin. Five sections of one or both
thyroids from each chicken and assay animal were mounted on
triplicate slides. Using a calibrated ocular micrometer, the thyroid
follicle exhibiting the highest epithelial cell in a section was used for
determining colloid and follicle diameter. A total of 18 measure-
ments were recorded for each chicken and 10 measurements for
each assay animal. These measurements were averaged and con-
verted to microns.
A hierarchical classification of the results from the chickens and bioassay animals was used for statistical evaluation. In the analysis of variance, the F test was used to determine significance of results. Asterisks adjacent to the standard errors of the mean (S.E.\textsubscript{m}) in the tables indicate that either the P is 0.01\textdagger\textdagger or 0.05\textdagger. The S.E.\textsubscript{m} were calculated from the error variance of the statistical analysis. The S.E.\textsubscript{m} is often followed by a number in parentheses which is the number of chickens represented in the sample when it differs from the total number of birds used in the group.

A statistical analysis was not performed on thyroid epithelial cell height or on colloid and follicle diameter in the assay animals. The methods used for analyzing these data were suggested by Dr. Kenneth E. Rowe, Department of Statistics. The statistical analysis was performed at the Computer Center of Oregon State University.
RESULTS

Comparison of Anatomical Measurements on Dwarf and Normal Chickens

A summary of anatomical measurements on dwarf and normal chickens 2-3 and 7-13 months of age is presented in Appendix Tables II and III. Statistical evaluation showed that normals were significantly heavier in body weight than dwarfs at these two ages. Because of the large differences in body weight and also the high S.E. \(_m\) weights of hypophyses, adrenals and thyroids relative to body weight have been presented. Normals within each age group exhibited significantly heavier absolute hypophysis weights than dwarfs. In contrast, the dwarfs 7-13 months of age yielded significantly heavier relative hypophysis weights than normals but no significant differences were found for this measurement between dwarfs and normals 2-3 months of age. Galvimetric measurements on the adrenals revealed that normals 2-3 months of age had significantly heavier glands than dwarfs on the basis of absolute weight but not on the basis of relative weight. In the 7-13 month age group, no significant differences in adrenal weights were obtained on an absolute weight basis, but on the other hand the dwarfs exhibited heavier adrenals than normals on a relative weight basis. Investigation of the reproductive system revealed that normals had significantly heavier ovarian
weights than dwarfs within the 2-3 month age group.

Comb weight and its related measurement - comb area - were significantly greater in normals than in dwarfs for the 7-13 month age group. No significant differences in comb size between normals and dwarfs were found in the 2-3 month age group. Shank length as indicative of body size was significantly greater for normals than for dwarfs within each age group.

Thyroid weights and histological studies on the thyroids of normals and of dwarfs are presented in Appendix Table IV. This investigation showed that normals had significantly heavier thyroids than dwarfs at both ages on a relative and an absolute weight basis. Histological examination of the thyroids revealed that normals 7-13 months of age secreted significantly more thyroxine than dwarfs of the same age as indicated by increased epithelial cell height. Epithelial cell height was almost identical in dwarfs and normals within the 2-3 month age group. Two additional measurements on the thyroids showed that normals exhibited significantly larger colloid and follicle diameter than dwarfs at both ages. Sections of the thyroids were more difficult to prepare for the normals than for the dwarfs in the older age classification. The colloid in the section had a tendency to wrinkle or else tear. Thyroid follicles from the older birds as compared to the younger birds were in many instances irregular in shape and the epithelium was broken and folded in many cases.
Bioassay of Chicken Hypophyses in the Hypophysectomized Rat

The responses from the bioassay of normal and dwarf whole chicken hypophyses in immature, hypophysectomized, female rats are presented in Appendix Tables V, VI, and VII.

**Somatotropic Hormone (STH)**

In the evaluation of STH activity, no significant differences in body weight gain, epiphyseal cartilage width and tail length were found between dwarfs and normals of each age group. As a check on species specificity to STH, the treated rats showed significantly greater body weight gains and epiphyseal plate responses than the controls. Also, the higher dosages of hypophyses produced greater body weight gains and epiphyseal plate responses than did the lower dosages of hypophyses. Of the three measurements, tail length was the most variable in response to dosage and no significant difference between the treated rats and the controls was found. Observation of the data on epiphyseal plate readings indicated that the younger birds had a higher hypophyseal STH content than the older birds.

**Hormones Affecting Thymic Activity**

Several hormones have been reported to stimulate the thymus
glands. No significant differences in thymic activity were found between dwarfs and normals in this bioassay. The treated rats produced a measurable but a non-significant higher thymic response than the controls.

**Adrenocorticotropic Hormone (ACTH)**

Based on adrenal weight of the assay rats, normals had a significantly higher hypophyseal ACTH content than dwarfs 2-3 months of age. On the other hand, no significant differences were found between dwarfs and normals 7-13 months of age. These findings showed that treated rats had significantly heavier adrenals than the controls. An age factor was observed with the younger birds exhibiting a higher ACTH content than the older birds.

**Gonadotropic Hormones (FSH and ICSH)**

In the determination of total gonadotropic activity, weights of the ovaries and oviducts and uteri of the assay rats were not significantly different between dwarfs and normals within each age group. These organs were significantly heavier in treated than in control rats. Greater responses were generally obtained from the higher dosages of hypophyseal homogenate than from lower dosages. Age of the chicken appears to play an important role in this portion of the investigation. According to ovary and oviduct and uterus weights,
the older birds are observed to have higher gonadotropic activity than the younger birds. Twice as many vaginal openings were produced in the rats by dwarfs as by normals 2-3 and 7-13 months of age. In regard to age, the younger birds induced 2 openings and the older birds induced 9 openings in the treated rats. No opening was found in the control rats.

Follicle-Stimulating Hormone (FSH)

Ovarian follicle diameter as a specific bioassay for FSH revealed that dwarfs did not differ significantly from normals within each age group. Rats receiving the hypophyseal homogenate did not differ significantly from the controls. However, a response was apparent at the highest hypophyseal dosage as compared to the lowest dosage. There is a slight indication that the younger birds have a higher hypophyseal FSH content than older birds.

Interstitial-Cell-Stimulating Hormone (ICSH)

Hypophyseal homogenate from dwarfs and from normals in each age group stimulated the interstitial cells in the rat ovary to about the same extent. The two highest dosages from normals and dwarfs produced either repair, partial repair or slight repair (deficient plus) of the interstitial cells. No interstitial cell stimulation was observed in the ovaries of the controls.
Thyroid-Stimulating Hormone (TSH)

The histological classification of the thyroids from the assay rats is represented in Appendix Table VII. Statistical evaluation of thyroid weight showed that the TSH content of hypophyseal homogenate from dwarfs and normals within each age group did not differ significantly. Also, the thyroids from the treated rats did not differ significantly from those of the controls. Due to loss of identity of the individual rat, statistical evaluation could not be performed; however, observation of these data reveals no differences between dwarfs and normals within each age group as to epithelial cell height or as to follicle and colloid diameter in the assay rats. A very slight increase in epithelial cell height may be observed in the rats receiving the highest dosages as compared to the controls. No appreciable difference between the treated rats and controls was obtained for colloid and follicle diameter.

Statistical evaluation of the responses in the assay rats to hypophyses from normal and dwarf White Leghorns is summarized in Table VIII.
DISCUSSION

Growth of Normal and Dwarf Chickens

The dwarfs are significantly smaller in body weight than normals at 2-3 months of age, which is in accord with Hutt (1949), van Tienhoven et al (1966) and Bernier and Arscott (1966). These data indicate that the dwarf grows at a slower rate than normals. These findings are in contrast to those observed in dwarf mice in which there is an almost complete cessation of growth at 17 to 35 days of age (Boettiger and Osborn, 1938).

Dwarfs had a significantly shorter shank than normals at both ages, which is in accord with Hutt (1949), Bernier and Arscott (1966) and van Tienhoven et al (1966). The tarso-metatarsus is the most sensitive of the three leg bones for measuring growth, which is controlled by heredity and influenced by environment. These data show only a 0.1 cm difference in shank length between normals and dwarfs of the different age groups. It may be postulated that the shank reaches its physiological limit in size at an early age. To support this postulate, van Tienhoven et al (1966) have shown that shank length increased by only 0.1 percent between 16 and 20 weeks of age in both normals and dwarfs.
Comb Weight and Comb Area

According to Dorfman and Shipley (1956), androgens are responsible for stimulating comb growth in male and female chickens. The chick comb method for the bioassay of androgens is discussed by Zarrow, Yochim and McCarthy (1964). The lack of a significant difference between dwarfs and normals 2-3 months of age in comb weight and area can be attributed to low gonadotropin secretion and consequently immature ovaries at this age. Since the ovaries of this age group are immature, androgen secretion would be very low; therefore, comb growth would be nearly the same for dwarfs and normals unless the dw gene was expressing its effect at this point. Within the 7-13 month age group, the normals had significantly larger combs than dwarfs, explained on the basis of larger body size provided androgen secretion is equal in relation to body size in normals and dwarfs. Dwarfs assume their places in the social organization with normals, indicating the same degree of stimulation by androgens.

Hypophysis and Its Hormone Content

The normals within each group had significantly heavier hypophyses than the dwarfs, which is similar to the findings of van Tienhoven et al. (1966) that the adenohypophyses of the normals
(K-line) were heavier than those of dwarfs from 1 day to 20 weeks of age. However, the dwarfs 7-13 months of age had heavier hypophyses relative to body weight than normals. The hypophysis weights in this investigation are assumed to be a measure of the hormone content of this gland.

In other words, it is assumed that bioassay of the hypophyses for total hormone content would reflect the physiological needs of the various endocrine glands under investigation and in turn these glands would reflect the secretory activity of the hypophysis. The important question, of course, is whether the hormone content of the hypophysis represents production, secretion or storage at the time this gland is collected. Ellington (1964) assumes that the hormone content of the hypophysis is the algebraic sum of synthesis, storage and secretion; therefore, hypophyseal hormone content would not have to reflect secretion under all conditions. Also, a reduced amount of a given hormone in the hypophysis does not always mean "consumption," making this phase of endocrinology very complex. At the present time, the best answer to the question is the use of plasma studies, thus measuring the amount of hormone secreted into the blood stream and serving the physiological needs of the organism.
Determination of Hormone Potency

Somatotropic Hormone (STH)

Van Tienhoven et al. (1966) conducted cytological studies on the adenohypophysis of sex-linked-recessive dwarf chickens and frequently found the occurrence of small cells with bilobed nuclei or with two nuclei. These cells contained secretory droplets which were speculated to be storage of STH because of a failure of its release into the blood stream. To follow up this finding, the present investigation showed that hypophyseal homogenate from dwarfs did not produce significantly greater growth response than normals in both age classifications, using the tibia test, body weight gain and tail length in rats.

The younger birds, disregarding type, produced greater epi-physeal plate responses than older birds, indicating a higher hypophyseal STH content during the growing period. Evidence to support this observation has been found by Hazelwood and Hazelwood (1961). These investigators, using 9-12-week-old White Rocks, not segregated as to sex, discovered that saline, 1/2, 1, 2 and 4 adenohypophyses produced tibia readings of 153±2, 163±2, 191±6, 236±5 and 285±15 micra, respectively, in hypophysectomized female rats.

Libby, Meites and Schaible (1955) and Glick (1960) have shown that STH from mammals has no effect on growth of young
chickens. The greater epiphyseal plate responses at the higher dosage levels in this investigation support the work of Solomon and Greep (1959) and Hazelwood and Hazelwood (1961), that large dosages of STH are needed to obtain measurable results in the rat. It appears that birds are less sensitive to mammalian STH than mammals are to avian STH. This finding may be attributed to the active STH core theory suggested by Wilhelmi (1955) and Li (1957). This theory is based on the evidence that an essential core of STH is present in somatotropin molecules of all species. This core is the biologically active portion of the molecule and the extra amino acids outside the core are responsible for species specificity. Therefore, the degree to which a species can strip the molecule to the active core will determine the response to STH.

**Thymus**

Thymus weight response in the rats did not reveal any difference between dwarf and normal chickens in each age classification. In the present bioassay, thymus weight varied with dosage. This finding may be supported by voluminous evidence. Hublé (1958) and Höhn (1961) report that adrenal cortical hormones cause regression of the thymus. Hublé also showed that testosterone and a mixture of estrogen and progesterone decreased thymus weight. In contrast, Höhn (1959) was unable to influence thymus weight in adult chickens
and ducks with testosterone. An increase in thymus weight was found after prolonged administration of thyroxine.

Increase in thymus weight has been reported as an assay for STH, according to Zarrow, Yochim and McCarthy (1964). Bruce, Parkes and Perry (1952), Thing (1953) and Thompson and Fisher (1953) have used thymus involution as an assay for ACTH. There appears to be no relationship of any particular hormone to thymic activity in this study. Since several hormones, as reported above, act upon the thymus, it would seem that the weight of this organ would vary to a certain extent.

**Adrenals and Adrenocorticotropic Hormone**

The diverging results between absolute and relative weights of the adrenals in these chickens, although not necessarily biologically unusual, may be attributed to environmental factors rather than to dwarfism. Stress, a word that covers many types of environmental changes, will increase adrenal weight. This phenomenon occurs through stimulation of the hypothalamus via the sensory limb causing hypersecretion of corticotropic releasing factor (CRF), consequently increasing ACTH output, thus increasing the weight of the adrenals. If one would rather rely on the heavier relative adrenal weight of the dwarfs, stress may play a large role at this point. Since the surface area per unit body weight of dwarfs is larger than
that of normals, environmental factors could have a greater effect on these small chickens.

Within the 2-3 month age group, the ACTH content of the hypophyses from normals stimulated the adrenals of the assay animals to a greater extent than hypophyses from dwarfs. Supporting evidence has been reported by Kamar and Rasek (1963) who found that the adrenal gland in chickens is a growth retarding factor. Dulin (1953) found that ACTH has a depressing effect on body weight of chickens. In addition, Marx, Simpson and Evans (1944) have reported that ACTH decreases the width of the epiphyseal plate in hypophysectomized rats. Based on these investigations in addition to the results from the present bioassay, it may be postulated that normals were storing ACTH; whereas, dwarfs were secreting ACTH, thus normals would have a larger body size than dwarfs.

An overall observation of these data revealed that hypophyses of younger birds increased adrenal weight to a greater degree than those of older birds. Again, on the basis of the above investigations, the young pullets were still growing; therefore, ACTH would be stored in the hypophysis and not secreted in significant amounts until the birds approach puberty.

A point of concern pertaining not only to adrenals but other endocrine glands is the secretion of hormones in other parts of the biological system rather than by a specific gland. According to
Brown, Brown and Meyer (1958), the adrenal glands in the chicken can function at a high level in the absence of the adenohypophysis. Sturkie (1964) reports that in several instances hypophysectomy did not cause a decrease in adrenal weight and that ACTH administration had very little effect on the adrenals. Nalbandov (1964) gives conclusive evidence that hypophysectomy without destruction of the stalk or median eminence allowed the adrenal steroid levels of the blood to remain significantly higher than in the control chickens. This indicates that an ACTH-like substance is being secreted by the stalk and median eminence in sufficient amounts to cause synthesis of adrenal steroids. Therefore, bioassay of tissue homogenate for a given hormone may not necessarily be a critical measurement since other portions or related tissues or even tissues in other parts of the body may secrete the same hormone.

Gonadotropic Hormones

In the evaluation of hypophyseal gonadotropic hormone content, no significant differences between dwarfs and normals were found. This is in accord with van Tienhoven et al. (1966) who found no differences in gonadotropic cells in the adenohypophyses of dwarf and normal chickens.

However, there appears to be a difference in gonadotropic potency due to age, disregarding type. Observation of these data
reveals that hypophyseal homogenate from the older birds produced heavier ovaries and oviducts and uteri and induced more vaginal openings in the rat than that from the younger birds. This is not in accord with Phillips (1942) who found that gonadotropic potency of chicken hypophyses was higher during the growing period and decreased with age. There seems to be a curvilinear relationship between age and hormone content as Breneman (1955) found that gonadotropic potency of hypophyses from female chickens increased from 20 to 126 days of age when evaluated by testicular response in chicks. Bacon, Cherms and McShan (1966) have shown a steady increase in gonadotropin concentration during the growing period of female turkeys. Therefore, the 7-13 month-old chickens could have a higher hypophyseal gonadotropin content than the 2-3 month-old birds.

Although sex differences in gonadotropic activity were not under investigation, a brief look at some findings from other workers may throw some light on the responses obtained in this study. According to Myer, Millish and Kupperman (1939), the adult male chicken has higher gonadotropic activity than the adult female chicken using rat ovarian weight response. Baily and Phillips (1952) found the gonadotropic potency of blood serum in laying females and adult males to be lower than in immature males, immature females and non-laying females. In support of these views, Riley
and Fraps (1942b), using the mouse uterine weight response, found that males 7-15 months of age have a gonadotropic potency eleven times greater than laying hens and seven times greater than non-laying hens. According to Domm (1931), who used comb development after hypophyseal implants, and according to Phillips (1942), who used vaginal cornification after administration of acetone-dried hypophyses, capons have higher gonadotropin potency than laying hens. Witschi, Stanley and Riley (1937) discovered no sex difference in turkeys as evaluated by rat ovary response which is in contrast to the findings in chickens. These turkeys were in their first season of sexual activity and it may be that their hypophyses contained less gonadotropins than those of older birds.

Turning now to two experiments involving gonadotropic potency in Japanese quail, *Coturnix coturnix japonica*, which illustrate the importance of time on the removal of the hypophysis as well as sex differences. Tanaka et al. (1965) investigated the effects of different light regimens on the gonadotropic activity of female Japanese quail. Bioassays of adenohypophyses using testicular weight response of 2-week-old quail revealed that birds exposed to continuous light had the highest gonadotropic activity and those exposed to 4 hours of light and 20 hours of darkness had the lowest activity. Bacon, Cherms and McShan (1964) found a greater gonadotropin content per hypophysis in male than in female Japanese quail.
In this investigation, the response of the ovarian follicles revealed that younger birds had a slightly higher hypophyseal FSH content. This would seem natural, since FSH would be expected to exist at high levels at this age in order to stimulate the ovarian follicles.

The study of the ICSH content in the hypophysis of chickens is very intriguing since Nalbandov, Myer and McShan (1951) have suggested the presence of a third gonadotropic hormone in this gland. Their conclusion was based on the finding that combs of male chickens, which atrophy after hypophysectomy, can be made to grow for 10 to 12 days after administration of mammalian ICSH, but the comb regresses in size thereafter in spite of continued administration of ICSH. However, continuous comb growth can be produced by administering gonadotropins from chickens. The present investigation did not reveal evidence for the presence of a third gonadotropin, but it did reveal that there is an ICSH factor in birds similar to that in mammals based on response of the reproductive system in the treated rats as compared to the controls.

There appears to be no difference between dwarfs and normals within each group with respect to ICSH content of the hypophyses. However, the 4 and 2 dosages of hypophyses produced the greatest amount of interstitial cell repair in the rat ovaries. In regard to age, no difference in ICSH activity was observed. A point of interest
regarding sex of the chicken; Riley and Fraps (1942a), using the African Weaver Finch test, found that the ICSH content was higher in males than in females.

Taking a brief look again at the chickens, the normals 2-3 months of age had significantly heavier ovarian weights than the dwarfs, which is in accord with van Tienhoven et al (1966). This is attributed to the larger body size of normals rather than an endocrine disturbance.

Luteotropic hormone (LTH), although secreted by the adeno-hypophysis in the fowl, has not been discussed in this study due to the fact it could not be evaluated critically in the present bioassay. The presence of LTH is reluctantly pointed out under "gonadotropins" because it has been found to be a luteotropic hormone only in rats, mice and possibly sheep. Since LTH does not act on the gonads of all species of animals, this hormone is thought not to be a gonadotropin (Nalbandov, 1964).

**Thyroids and Thyroid-Stimulating Hormone**

Upon examination of the chickens, the normals had significantly heavier thyroids than dwarfs which is in accord with van Tienhoven et al (1966). These same differences existed when thyroid weight was related to body weight. Based upon epithelial cell height, dwarfs and normals 2-3 months of age secrete the same
amount of thyroxine. At 7-13 months of age, dwarfs appear to be secreting less thyroxine than normals. The thyroids in the dwarfs exhibited significantly smaller colloid and follicle diameter than normals within each age group. This is similar to the findings of van Tienhoven et al. (1966) who found that normals had a significantly higher percentage of colloid than dwarfs from day-old to 20 weeks of age.

This investigation revealed that the TSH content of hypophyseal homogenate from dwarfs and normals is about the same as indicated by thyroid weight response in the rat. The treated rats did not differ from the controls. Epithelial cell height, and colloid and follicle diameter were similar for dwarfs and for normals in each age classification. The epithelial cells in the thyroids from the rats receiving the highest dosages appear slightly taller than those from the thyroids of the control rats. No differences between the treated rats and the controls in colloid and follicle diameter were observed. There is no conclusive evidence, but these data indicate a species specificity to TSH. In support of this evidence, Leonard (1938) did not obtain a response in the thyroid of rats receiving acetone-dried avian hypophyses.

Histological examination of the thyroid may not always be indicative of its secretory activity (Barrington, 1963). In support of this view, Grollman (1947) reports that response which occurs in one follicle may not occur in an adjacent one and only certain cells
in a follicle may be affected.

Certain internal and external characteristics would indicate that the \underline{dw} gene is causing a hypothyroid condition. The fact that \textit{van Tienhoven et al} (1966) and the present study revealed that thyroids from dwarfs are smaller than those of normals is not conclusive proof of hypothyroidism. According to Sturkie (1964), enlarged thyroids do not reflect either a hyper- or hypofunctioning gland.

Taller epithelial cells in the thyroids of normals compared to dwarfs 7-13 months of age would indicate hypothyroidism in the latter. The bioassay of hypophyseal homogenate did not confirm the above results, although the rat may be unresponsive to avian TSH. According to Frey and Flock (1958), the TSH content of hypophyses from the day-old chick is very low; therefore, a higher dosage of hypophyseal homogenate may be needed for response in the rat from the younger birds. The smaller follicle and colloid diameter of dwarfs compared to normals within each age group suggest hyper-secretion by the former. Although not always the case, it would seem that since the thyroids of the dwarfs are smaller than those of normals that the follicle and colloid diameter would also be smaller. In other words, the relationship between thyroid weight and colloid and follicle diameter is of concern.

The dwarfs under study are less temperamental than normals.
In a few cases, the feathers of younger birds are "lacy." These dwarfs have been observed to begin molting later than normals. These three factors could be attributed to hypothyroidism. Sturkie (1964) reports that hypothyroidism reduces the length of the long bones and the size of the skeleton, which has been found to be characteristic of the sex-linked-recessive dwarf. Grollman (1947) reports that calcium retention is caused by hypothyroidism. An increased amount of calcium is required in the ration of sex-linked dwarfs according to Bernier and Arscott (1966). A large amount of abdominal fat also accumulates in the dwarf chicken with increasing age. This may be explained by Turner (1948) and Sturkie (1964) who report that lipids in the blood and as a consequence obesity results from depressed thyroxine secretion.

The onset of egg production is only slightly delayed in the dwarf, indicating a satisfactory thyroid secretion rate. Egg production is nearly as high as in the normals.

These dwarfs exhibit protrusion of the eyeballs, characteristic of exophthalmic goiter in man, due to a hyperthyroid condition. Several investigators have provided evidence for this condition. Höhn (1961) reports that TSH per se is responsible for causing exophthalmus in mammals and has also been shown to produce the same condition in ducklings. Schockaert (1931) found that hypophyseal extracts cause exophthalmia in ducks. According to Turner
(1948), several theories have been proposed for eyeball protrusion. The deposition of excessive amounts of fat behind the eyeball, congestion of blood vessels behind the eye and swelling of the extraocular muscles have been proposed. Dobyns and Steelman (1953) have reported on an exophthalmic producing substance (EPS) and Condliffe (1963) has reported on an exophthalmogenic factor (EF) both of which are different from TSH.

Van Tienhoven et al (1966) were unable to correct the dwarf condition when feeding protamone to dwarfs from one day to eight weeks of age, which is an indication of an adequate TSH and thyroxine secretion. Ovary and oviduct weights were not affected by protamone feeding. Cytological studies of the adenohypophysis revealed no differences in thyrotropic cells in dwarfs and in normals. A point of concern here is that humans suffering from a hypothyroid condition are more sensitive to thyroxine administration than humans with normal thyroids (Grollman, 1947). According to Turner (1948), there is evidence that thyroxine is necessary for the secretion of STH. The STH content of hypophyses from dwarfs in this study was found to be about equal to that of normals, indicating an adequate thyroid secretion rate.

**Neurohypophyseal Hormones**

The hormones of the neurohypophysis (posterior pituitary)
have not been investigated in this study because critical measurements of their physiological activity could not be evaluated. There is much contrasting evidence among investigators at the present time as to the types of hormones stored in the neurohypophysis. A recent study by Munsick (1964), using ion exchange chromatography, revealed the presence of oxytocin and arginine vasotocin in neurohypophyseal extracts from chickens and turkeys.

The question still remains as to which endocrine gland/glands is/are associated with dwarfism. A more sensitive test for determining the secretory activity of the thyroid, such as the use of radioiodine studies, may answer the question. Investigation of the parathyroid may be in order, since dwarfs require more calcium in their ration than normals. There is one area that might provide a clue and this is the effect of the thyroid on the nervous system. Zarrow, Yochim and McCarthy (1964) state that the young hypothyroid animal (dwarf) exhibits retardation in the development of the nervous system associated with an impairment in the formation of myelin sheaths. The nervous system may, in part, be responsible for the quieter disposition of the dwarfs. Turner (1948) reports that exophthalmia can be produced experimentally by stimulation of the sympathetic nerves which innervate the orbitalis muscle of the eye.
SUMMARY

A comparison of various endocrine glands, in addition to other anatomical measurements, was made on 19 normal and 21 dwarf 2-3-month old females and 20 normal and 20 dwarf 7-13-month old females of the White Leghorn breed. At both ages, dwarfs weighed less and had shorter shanks than normals. Comb weight and comb area (L X H) were larger in normals than in dwarfs 7-13 months of age, but no difference was revealed in the 2-3 month age group. The lack of difference in comb size is attributed to a low rate of secretion of androgen at this young age. Ovaries in 2-3 month age group were heavier in normals than in dwarfs due to greater gonadotropin secretion in relation to body size. The divergent results in weight of the hypophyses on an absolute and a relative-to-body-weight basis, although not necessarily biologically unusual, may be attributed to the algebraic sum of synthesis, storage and liberation of the total number of hormones by this gland. Also, absolute and relative weights of the adrenals did not always coincide in both age groups, which may be attributed to environmental factors influencing the release of ACTH from the hypophyses prior to removal of this gland, rather than dwarfism. The normals had heavier thyroids on an absolute and on a relative-to-body weight basis at both ages than dwarfs, which does not necessarily
indicate a hyper- or hypothyroid condition in the latter. Histological studies on the thyroids revealed that normals were secreting more thyroxine than dwarfs at 7-13 months of age, based on epithelial cell height. There was no difference between normals and dwarfs 2-3 months of age in regards to this measurement. The smaller colloid and follicle diameter found in thyroids of dwarfs than of normals may be attributed to the lower gland weights in the latter group.

Bioassay of the hypophyses from these chickens was conducted in immature, hypophysectomized, female rats. Dwarfs did not differ from normals in STH content of the hypophysis, according to the tibia test, body weight gain and tail length. Greater epiphyseal plate response was obtained from the hypophyses of growing birds. Thymus weight varied with dosage, which is attributed to several hormones acting on this gland. In the younger age group, the ACTH content of the hypophyses of normals produced heavier adrenals in the rat than that of dwarfs, which is believed to be due to the hormone content of the hypophyses at the time of removal, rather than to dwarfism. The younger birds appeared to have a higher ACTH content than older birds, due to storage of this hormone in the hypophysis at this age. Dwarfs did not differ from normals in gonadotropinic activity, as evaluated by ovary and oviduct and uterus weight responses. However, the older birds appeared to
have a higher hypophyseal gonadotropin content than the younger birds, based on ovary, oviduct and uterus weights and vaginal openings. The FSH content, as evident by follicle diameter, was found to be the same in dwarfs as in normals at both ages. The evaluation of ICSH revealed no difference between dwarfs and normals at both ages, but a striking response was observed at the 4 and 2 dosages of hypophyses.

There was no difference in TSH content between dwarfs and normals at either age, as determined by thyroid weight in the assay rat. Histological characteristics of the thyroids revealed no difference between dwarfs and normals using epithelial cell height and colloid and follicle diameter. Except for slightly taller epithelial cells at the higher dosages, colloid and follicle diameter of the treated rats did not differ from the controls. Also, the treated rats did not differ from the controls in thyroid weight. These findings indicate that the thyroids in the rat are very sensitive to avian TSH.

Even though the precise cause of sex-linked-recessive dwarfism in chickens was not found in the present investigation, different findings and theories have been presented to stimulate thought and lay the foundation for further investigations, such as, the use of radio-iodine studies. The parathyroid and the thyroid in relation to the nervous system also need investigation.


Appendix Table I.  Growth and feed requirements of dwarf and normal pullets from day-old to 23 weeks of age.

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>Body Weight</th>
<th>Cumulated Feed Consumption</th>
<th>Feed Conversion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dwarf (gm)</td>
<td>Normal (gm)</td>
<td>% of Normal</td>
</tr>
<tr>
<td>4</td>
<td>218</td>
<td>289</td>
<td>75.4</td>
</tr>
<tr>
<td>8</td>
<td>460</td>
<td>641</td>
<td>71.8</td>
</tr>
<tr>
<td>12</td>
<td>706</td>
<td>1006</td>
<td>70.2</td>
</tr>
<tr>
<td>17</td>
<td>896</td>
<td>1224</td>
<td>73.2</td>
</tr>
<tr>
<td>23</td>
<td>1029</td>
<td>1464</td>
<td>70.3</td>
</tr>
</tbody>
</table>

1. \( \frac{\text{Dwarf}}{\text{Normal}} \times 100 \).

From Bernier and Arscott, 1966.  Unpublished data, Oregon Agricultural Experiment Station.
### Appendix Table II. Comparison of organ weights in normal and dwarf White Leghorns.

<table>
<thead>
<tr>
<th>Chickens Type</th>
<th>No.</th>
<th>Age (months)</th>
<th>Body Weight (gm)</th>
<th>Hypophysis Weight</th>
<th>Adrenal Weight</th>
<th>Ovary Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>19</td>
<td>2-3</td>
<td>970 ± 34**</td>
<td>5.9 ± 0.4**</td>
<td>79.7 ± 5.3*</td>
<td>8.3 ± 0.5</td>
</tr>
<tr>
<td>Dwarf</td>
<td>21</td>
<td>2-3</td>
<td>716 ± 27</td>
<td>4.2 ± 0.4 (19)</td>
<td>61.7 ± 5.0</td>
<td>8.8 ± 0.5</td>
</tr>
<tr>
<td>Normal</td>
<td>20</td>
<td>7-13</td>
<td>1582 ± 34**</td>
<td>8.4 ± 0.4* (19)</td>
<td>135.9 ± 5.4(18)</td>
<td>8.6 ± 0.5</td>
</tr>
<tr>
<td>Dwarf</td>
<td>20</td>
<td>7-13</td>
<td>1148 ± 50</td>
<td>7.0 ± 0.4 (19)</td>
<td>121.1 ± 5.6(17)</td>
<td>10.9 ± 0.5**(17)</td>
</tr>
</tbody>
</table>

( ) Number in parentheses represents the number of chickens in the sample when different from the number in the second column.

** P < 0.01
* P < 0.05
Appendix Table III. Comparison of the comb and shank in normal and dwarf White Leghorns.

<table>
<thead>
<tr>
<th>Type</th>
<th>No.</th>
<th>Age (months)</th>
<th>Weight (gm)</th>
<th>Area (L X H) (cm²)</th>
<th>Shank Length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>19</td>
<td>2-3</td>
<td>0.57 ± 0.64</td>
<td>4.9 ± 1.6</td>
<td>9.5 ± 0.1*(18)</td>
</tr>
<tr>
<td>Dwarf</td>
<td>21</td>
<td>2-3</td>
<td>0.36 ± 0.61</td>
<td>3.5 ± 1.6</td>
<td>7.8 ± 0.1</td>
</tr>
<tr>
<td>Normal</td>
<td>20</td>
<td>7-13</td>
<td>11.6 ± 0.6**</td>
<td>38.0 ± 1.6**</td>
<td>9.4 ± 0.1***(10)</td>
</tr>
<tr>
<td>Dwarf</td>
<td>20</td>
<td>7-13</td>
<td>7.5 ± 0.6</td>
<td>28.4 ± 1.6</td>
<td>7.9 ± 0.1</td>
</tr>
</tbody>
</table>

( ) Number in parentheses represents the number of chickens in the sample when different from the number in the second column.

** P < 0.01
*   P < 0.05
### Appendix Table IV. Histological characteristics of the thyroids in normal and dwarf White Leghorns.

<table>
<thead>
<tr>
<th>Chickens Type</th>
<th>No.</th>
<th>Age (months)</th>
<th>Absolute Weight (mg)</th>
<th>Relative Weight (mg/100 gm of body wt)</th>
<th>Epithelial Cell Height (μ)</th>
<th>Colloid Diameter (μ)</th>
<th>Follicle Diameter (μ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>19</td>
<td>2-3</td>
<td>86.7 ± 8.2**(18)</td>
<td>8.7 ± 0.5* (18)</td>
<td>11.9 ± 0.4</td>
<td>96.4 ± 5.2**</td>
<td>130.5 ± 6.2**</td>
</tr>
<tr>
<td>Dwarf</td>
<td>21</td>
<td>2-3</td>
<td>49.9 ± 7.6</td>
<td>6.9 ± 0.5</td>
<td>11.3 ± 0.4</td>
<td>76.5 ± 4.9</td>
<td>104.5 ± 5.9</td>
</tr>
<tr>
<td>Normal</td>
<td>20</td>
<td>7-13</td>
<td>168.3 ± 7.8**</td>
<td>10.6 ± 0.5**</td>
<td>10.3 ± 0.5**(12)</td>
<td>138.0 ± 6.5***(12)</td>
<td>178.6 ± 7.8***(12)</td>
</tr>
<tr>
<td>Dwarf</td>
<td>20</td>
<td>7-13</td>
<td>52.0 ± 9.0 (15)</td>
<td>4.5 ± 0.6 (15)</td>
<td>7.7 ± 0.4 (18)</td>
<td>75.0 ± 5.3 (18)</td>
<td>98.7 ± 6.4 (18)</td>
</tr>
</tbody>
</table>

( ) Number in parentheses represents the number of chickens in the sample when different from the number in the second column.

** P < 0.01  
* P < 0.05
Appendix Table V. Responses in immature, hypophysectomized, female rats to hypophyses from normal and dwarf White Leghorns.

<table>
<thead>
<tr>
<th>Type</th>
<th>Age (months)</th>
<th>Dosage (No. of Hypophyses)</th>
<th>Body Weight Gain (gm)</th>
<th>Epiphyseal Width (µ)</th>
<th>Tail Length (cm)</th>
<th>Thymus Weight (mg)</th>
<th>Adrenal Weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>--- (Control) 13</td>
<td>0</td>
<td>2.5±0.8</td>
<td>147±11</td>
<td>11.6±0.2</td>
<td>217±18</td>
<td>8.7±0.8</td>
<td></td>
</tr>
<tr>
<td>Normal 2-3</td>
<td>2</td>
<td>4</td>
<td>8.5±2.0</td>
<td>270±29</td>
<td>13.7±0.4</td>
<td>314±45</td>
<td>18.4±2.1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2</td>
<td>5.5±2.0</td>
<td>221±29</td>
<td>11.4±0.4</td>
<td>236±45</td>
<td>12.3±2.1</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1</td>
<td>6.3±1.7</td>
<td>210±24</td>
<td>11.7±0.3</td>
<td>273±37</td>
<td>10.6±1.7</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1/4</td>
<td>4.0±1.7</td>
<td>219±24</td>
<td>12.1±0.3</td>
<td>261±37</td>
<td>15.3±1.7</td>
</tr>
<tr>
<td>Dwarf 2-3</td>
<td>2</td>
<td>4</td>
<td>12.0±2.0</td>
<td>270±29</td>
<td>11.3±0.4</td>
<td>286±45</td>
<td>13.5±2.1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2</td>
<td>7.0±1.7</td>
<td>222±24</td>
<td>11.5±0.3</td>
<td>303±37</td>
<td>10.2±1.7</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1</td>
<td>6.3±1.7</td>
<td>225±24</td>
<td>12.3±0.3</td>
<td>285±37</td>
<td>10.5±1.7</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1/4</td>
<td>3.3±1.7</td>
<td>181±24</td>
<td>11.8±0.3</td>
<td>223±37</td>
<td>9.5±1.7</td>
</tr>
<tr>
<td>Normal 7-13</td>
<td>2</td>
<td>4</td>
<td>10.0±2.0</td>
<td>209±29</td>
<td>12.6±0.4</td>
<td>259±45</td>
<td>9.1±2.1</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2</td>
<td>8.0±1.7</td>
<td>192±24</td>
<td>11.5±0.3</td>
<td>273±37</td>
<td>12.0±1.7</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1</td>
<td>7.0±1.7</td>
<td>191±24</td>
<td>12.0±0.3</td>
<td>300±37</td>
<td>9.9±1.7</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1/4</td>
<td>2.4±1.7</td>
<td>174±24</td>
<td>11.9±0.3</td>
<td>222±37</td>
<td>9.4±1.7</td>
</tr>
<tr>
<td>Dwarf 7-13</td>
<td>2</td>
<td>4</td>
<td>9.5±2.0</td>
<td>203±29</td>
<td>12.4±0.4</td>
<td>307±45</td>
<td>8.8±2.1</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2</td>
<td>8.3±1.7</td>
<td>179±24</td>
<td>11.9±0.3</td>
<td>233±37</td>
<td>8.7±1.7</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1</td>
<td>8.3±1.7</td>
<td>239±24</td>
<td>11.5±0.3</td>
<td>224±37</td>
<td>8.9±1.7</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1/4</td>
<td>5.3±1.7</td>
<td>183±24</td>
<td>11.4±0.3</td>
<td>159±37</td>
<td>7.6±1.7</td>
</tr>
</tbody>
</table>
### Appendix Table VI. Responses in the reproductive system of immature, hypophysectomized female rats to hypophyses from normal and dwarf White Leghorns.

<table>
<thead>
<tr>
<th>Chickens Type</th>
<th>Dosage (No. of Hypophyses)</th>
<th>Ovary Weight (mg)</th>
<th>Ovarian Follicle Diameter ($\mu$)</th>
<th>Oviduct and Uterus Weight (mg)</th>
<th>Vaginal Opening$^1$</th>
<th>Interstitial Cell Repair$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>--- (Control)</td>
<td>13 0</td>
<td>7.7$\pm$0.5</td>
<td>371$\pm$11</td>
<td>18.2$\pm$1.5</td>
<td>0 13</td>
<td>0 13</td>
</tr>
<tr>
<td>Normal 2-3</td>
<td>2 4</td>
<td>7.5$\pm$1.4</td>
<td>432$\pm$28</td>
<td>29.6$\pm$3.9</td>
<td>0 2</td>
<td>2 0</td>
</tr>
<tr>
<td></td>
<td>2 2</td>
<td>10.5$\pm$1.4</td>
<td>421$\pm$28</td>
<td>23.4$\pm$3.9</td>
<td>0 2</td>
<td>2 0</td>
</tr>
<tr>
<td></td>
<td>3 1</td>
<td>9.9$\pm$1.1</td>
<td>423$\pm$23</td>
<td>21.3$\pm$3.2</td>
<td>0 3</td>
<td>2 1</td>
</tr>
<tr>
<td></td>
<td>3 1/4</td>
<td>6.5$\pm$1.1</td>
<td>406$\pm$23</td>
<td>20.7$\pm$3.2</td>
<td>0 3</td>
<td>1 2</td>
</tr>
<tr>
<td>Dwarf 2-3</td>
<td>2 4</td>
<td>9.4$\pm$1.4</td>
<td>468$\pm$28</td>
<td>22.1$\pm$3.9</td>
<td>0 2</td>
<td>2 0</td>
</tr>
<tr>
<td></td>
<td>3 2</td>
<td>7.2$\pm$1.1</td>
<td>381$\pm$23</td>
<td>19.6$\pm$3.2</td>
<td>0 3</td>
<td>3 0</td>
</tr>
<tr>
<td></td>
<td>3 1</td>
<td>6.3$\pm$1.1</td>
<td>395$\pm$23</td>
<td>21.6$\pm$3.2</td>
<td>1 2</td>
<td>3 0</td>
</tr>
<tr>
<td></td>
<td>3 1/4</td>
<td>6.7$\pm$1.1</td>
<td>368$\pm$23</td>
<td>17.9$\pm$3.2</td>
<td>1 2</td>
<td>0 3</td>
</tr>
<tr>
<td>Normal 7-13</td>
<td>2 4</td>
<td>11.2$\pm$1.4</td>
<td>390$\pm$28</td>
<td>55.1$\pm$3.9</td>
<td>1 1</td>
<td>2 0</td>
</tr>
<tr>
<td></td>
<td>3 2</td>
<td>10.5$\pm$1.1</td>
<td>392$\pm$23</td>
<td>36.0$\pm$3.2</td>
<td>1 2</td>
<td>3 0</td>
</tr>
<tr>
<td></td>
<td>3 1</td>
<td>9.1$\pm$1.1</td>
<td>374$\pm$23</td>
<td>23.7$\pm$3.2</td>
<td>0 3</td>
<td>0 3</td>
</tr>
<tr>
<td></td>
<td>3 1/4</td>
<td>8.6$\pm$1.1</td>
<td>361$\pm$23</td>
<td>22.3$\pm$3.2</td>
<td>1 2</td>
<td>0 3</td>
</tr>
<tr>
<td>Dwarf 7-13</td>
<td>2 4</td>
<td>14.6$\pm$1.4</td>
<td>390$\pm$28</td>
<td>62.5$\pm$3.9</td>
<td>1 1</td>
<td>2 0</td>
</tr>
<tr>
<td></td>
<td>3 2</td>
<td>10.5$\pm$1.1</td>
<td>402$\pm$23</td>
<td>36.7$\pm$3.2</td>
<td>2 1</td>
<td>3 0</td>
</tr>
<tr>
<td></td>
<td>3 1</td>
<td>10.7$\pm$1.1</td>
<td>361$\pm$23</td>
<td>24.1$\pm$3.2</td>
<td>3 0</td>
<td>2 1</td>
</tr>
<tr>
<td></td>
<td>3 1/4</td>
<td>7.7$\pm$1.1</td>
<td>343$\pm$23</td>
<td>20.2$\pm$3.2</td>
<td>0 3</td>
<td>1 2</td>
</tr>
</tbody>
</table>
Appendix Table VI. Continued.

1. + = Number of rats with open vagina.
   - = Number of rats with closed vagina.

2. + = Number of rats showing interstitial cell response.
   - = Number of rats not showing interstitial cell response.

   + indicates deficient plus, partially repaired, repaired or hypertrophy.
   - indicates deficient.
Appendix Table VII. Histological characteristics of the thyroids in the assay rats.

<table>
<thead>
<tr>
<th>Chickens Type</th>
<th>Age (months)</th>
<th>Dosage (No. of Hypophyses)</th>
<th>Thyroid Weight (mg)</th>
<th>Epithelial Cell Height (μ)</th>
<th>Colloid Diameter (μ)</th>
<th>Follicle Diameter (μ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>2-3</td>
<td>2</td>
<td>7.0±1.1</td>
<td>8.0±0.5</td>
<td>50.5±4.6</td>
<td>73.1±5.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>4.0±1.1</td>
<td>7.9±0.5</td>
<td>51.1±4.6</td>
<td>73.2±5.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>6.0±0.9</td>
<td>7.5±0.4</td>
<td>49.9±3.8</td>
<td>74.8±4.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1/4</td>
<td>5.9±0.9</td>
<td>6.9±0.4</td>
<td>51.0±3.8</td>
<td>72.9±4.1</td>
</tr>
<tr>
<td>Dwarf</td>
<td>2-3</td>
<td>2</td>
<td>4.3±1.1</td>
<td>8.4±0.5</td>
<td>48.3±4.6</td>
<td>75.0±5.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>4.5±0.9</td>
<td>6.1±0.4</td>
<td>48.3±3.8</td>
<td>69.2±4.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>6.3±0.9</td>
<td>6.6±0.4</td>
<td>57.8±3.8</td>
<td>79.3±4.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1/4</td>
<td>4.8±0.9</td>
<td>6.8±0.4</td>
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Appendix Table VIII. Statistical evaluation of the responses in immature, hypophysectomized, female rats to hypophyses from normal and dwarf White Leghorns.

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<th>Comparison</th>
<th>Chickens Age (months)</th>
<th>Body Weight Gain (gm)</th>
<th>Epi-physeal Width (µ)</th>
<th>Tail Length (cm)</th>
<th>Thymus Weight (mg)</th>
<th>Adrenal Weight (mg)</th>
<th>Ovary Weight (mg)</th>
<th>Ovarian Follicle Diameter (µ)</th>
<th>Oviduct &amp; Uterus Weight (mg)</th>
<th>Thyroid Weight (mg)</th>
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NS = Not significant